FT-NIR SPECTROSCOPY OF FRESH AND TREATED MUSCLE TISSUE IN YOUNG FEMALE RABBITS

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ABSTRACT

A total of 32 young female rabbits (live weight 3.648 g) were made to fast or allowed to eat for 36 h before slaughter. Samples of the *Longissimus lumborum* (LL) muscle and two strips of the *Obliquus abdominis* muscle (OA) were sampled from chilled carcasses. The muscles were divided into three parts: 1) to determine the moisture; 2) placed in a plastic tube, then immersed in 95% ethanol; 3) stored at -18 ℃, then freeze-dried. A further sample, from the dissected hindleg (HL), was stored at -18 °C and freeze-dried. The quality traits were measured in raw LL, cooked or alcohol treated. An interesting close relationship (r=0.78) emerged between the two substrates used for shear force measurements: alcohol *vs.* cooked. The muscles were submitted to FT-NIR spectroscopy as fresh tissue (LL, OA and HL), then as intact freeze-dried (LL_if and HL_if) and also as ground freeze-dried (LL_gf, OA_gf and HL_gf), and were then used to predict the intramuscular lipid content. Two intact alcohol samples (LL_e and OA_e) were examined. The spectra were correlated and cross-validated to fixed experimental effects as binary data and to quantitative traits. 1-VR cross-validated values were reported. The fasting effect shown by the NIR was almost four times lower than the replication effect (av.ge 1-VR = 0.12 *vs* 0.52). The freeze-drying of substrates amplified the effects in the NIR spectra, while the alcohol treatment generally decreased them. The estimation of the intramuscular lipid content through scanning of the intact muscles was nearly half-efficient *vs* scanning of the ground freeze-dried tissue for OA (0.55) and for HL (0.53), but was poor for LL (0.16). An agreement between the spectra of OA and the presence of noising and experimental effects was confirmed. The LL in ethanol, which is easy to transport to a laboratory, seems to be suitable for tenderness measurements, but the NIR scan should be further investigated for meat quality assessment.

Key words: rabbit, muscle, FT-NIR, fasting, meat quality.

INTRODUCTION

Over the last decade many attempts have been made to show the usefulness of NIR spectroscopy applied to preliminary analyses of biological tissue from experimental rabbits (MASOERO *et al*., 1994; MASOERO *et al*., 2003). The most limiting factors for an "analytical" use were the noising due to replication effects, the great influence of the

water in the tissue and the lack of specific peaks of the substances in the NIR spectra of complex matrices such as muscle, liver or blood tissues. On the other hand, we have always utilised the NIR spectroscopy as a "synthetic" tool because it is a real predictor of significant effects even before the analyses have been carried out. In a companion paper (MASOERO *et al*, 2004), we have examined NIR scans of the plasma of experimentally fasted or non-fasted rabbits. In this paper, while dealing with the same rabbits, we aim to check the FT-NIR correlation with some biological traits of fresh and differently treated muscles. This is the first time we have directly examined muscle tissue. A completely new preparation was used, by immersion in 95% ethanol, a system that could easily be expanded in the field, to replace the cold-chain method, for the simple collection, accurate storage and easy transportation of samples.

MATERIAL AND METHODS

The trial was carried out in two replications using a total of 32 hybrid females of live weight 3648 ± 365 g. Eight rabbits were made to fast for each repetition. Before the final fasting started, the rabbits were weighed then fasted for a 36-hour period. As a control group, the other animals were fed 150 g/d of a commercial diet (crude protein 16.5%, crude fibre 14.0%) until slaughtering occurred. Carcasses chilled for 24 hours at 4 °C were examined according to harmonised procedures. The right *Longissimus lumborum* (LL) muscle and two strips of muscle cut around the *linea alba* of *Obliquus abdominis* (OA) were sampled from each carcass. Both the LL and OA muscles were divided into three portions. The moisture was determined on the first share using the standard analysis method (105 °C for 24 hours). The second share was placed in a plastic tube, like that for milk analysis, immersed in 95% ethanol and analysed for the dry matter content, after a two-day storage period. The third one was stored at -18 °C, then it was weighed and freeze-dried to determine the sample water lost during the freeze-drying process. After dissection and separation of the hindleg (HL) into meat and bone, a meat sample was minced and stored at -18 °C until freeze-drying was carried out, so as to calculate the water content by freeze-drying. The pH was measured on the fresh left LL using a Crison 507 pH-meter, with a Crison spear electrode and an automatic temperature compensator. The L* a* b* colour, chroma (C*) and hue (H*) indexes were determined, according to the CIElab system, using a Minolta CR331C Chromameter. The cooking losses of LL were measured after 30' at 70 °C in a water bath. The shear force (kg/cm^2) was recorded on a 0.5' carrot orthogonal to the fibres, using an Instron equipped with a Warner-Bratzler device, both on the LL cooked sample and on the LL portion stored with ethanol for two days. The intramuscular lipid contents were estimated using the previous NIR specific equations, already used by MASOERO *et al*., 2000, but now recalibrated to the spectra scanned by the P.E. Spectrum IdentiChek FT-NIR System (Perkin-Elmer, Beaconsfield, England; 1000-2500 nm, 3001 points), then chemometrised by the Spectrum Quant+ software with the standard PLS1 method and a mathematical treatment 1,5,5,1. The muscles were examined by FT-NIR spectroscopy as fresh trimmed tissue (LL, OA and HL), then as intact freeze-dried (LL_if and HL_if) and also as ground freeze-dried (LL_gf, OA_gf and HL_gf). The LL and OA intact samples were also examined after ethanol treatment (LL_e and OA_e). All these samples were directly submitted, in the trans-reflectance mode, to FT-NIR spectroscopy

using the Spectrum IdentiCheck FTNIR System. The spectra were imported into the NIRS-2 software, then mathematically treated as 1,5,5,1 with SNV and Detrend, and calibrated with an MPLS method set to 2 passages for outlier elimination with a liberal t-level (2.00), standard X (10) and H (10) values. Cross-validation was performed on 25 subgroups and the statistic parameter retained from this software was the 1-VR, corresponding to an R^2 in the validation mode. The fitted values were binary data for the replication and fasting, and quantitative for the biological variables. A principal component analysis of the 22 variables, included the replication and fasting, was performed with the PROC PRINCOMP of the SAS System.

RESULTS AND DISCUSSION

The results for the recalibration of the lipid content are shown in Table 1. As expected, these compounds were clearly perceived and accurately predicted in the LL, OA and HL sites. The high level of explained variance derives from an accurate pre-selection of the analysed samples, which were cut off from some 878 scanned rabbits.

Table 1. Calibration of the lipid content of LL, OA and HL muscles

Tissue	N LV	% Variance	SEP	Mean	N. of samples
LL	5	99.08	0.48	3.65	73
OA	4	99.58	1.18	22.0	44
HL	4	98.93	0.90	10.6	55

N LV = Number of Latent Variables; SEE = Standard Error of Estimate; SEP Standard Error of Prediction

The abscissa and ordinate axes in Figure 1 report the vectors of the first two principal components, which represented 30.2% and 14.6% of the variance, respectively, of the 22 variables analysed. As expected, and in spite of the different measurement systems, the water content of the muscles appeared closely grouped together and they were linked to the higher pH values, induced by the fasting treatment. On the opposite side of the figure, the lipid content of the three muscles appears grouped together. It should be pointed out that the lipid was expressed as content on a dry matter basis, but the relations with water appeared to be high and negative because of ontogeny.

Figure 2 plots the relation between the substrates (alcohol *vs* cooked) that were used for the shear force measurements, relative to the 16 samples from the first replication; the best fit (R^2=0.60; r=0.78) was obtained from the following exponential equation ($y=1.6009\ e^{0.2037\ X}$). The second replication did not give such good results, probably because the technique had been changed, collecting samples of ∅ 0.5', instead of 1.0', which were too small for alcohol immersion in the tube.

The previous companion paper (MASOERO *et al*, 2004), showed that the replication effect was more pronounced than the fasting effect, according to the analyses carried out for many biological traits. When the FT-NIR spectra of the muscles were fitted to the variables of the experimental design, the same general tendency emerged (Table 2).

The fasting effect was almost four times smaller than the replication effect (average 1-VR= 0.12 *vs* 0.52). It appeared at its highest in freeze-dried HL when it was examined intact (0.42), but it disappeared when it was ground (Table 2).

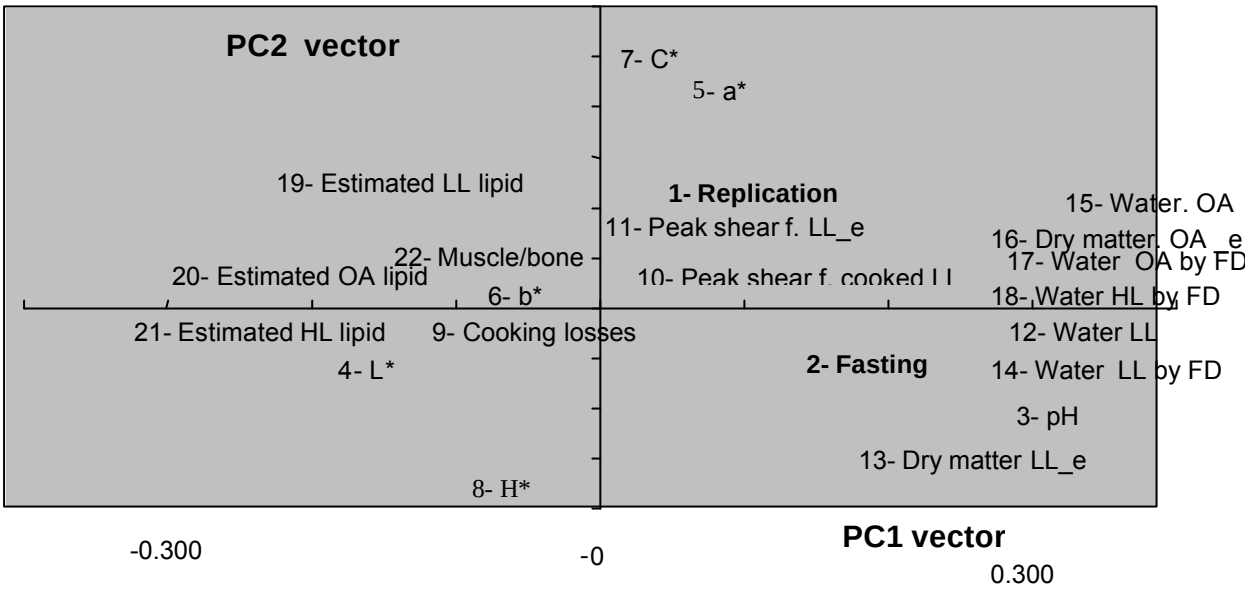

Figure 1. Plots of 22 analysed variables obtained from Principal Component Analysis

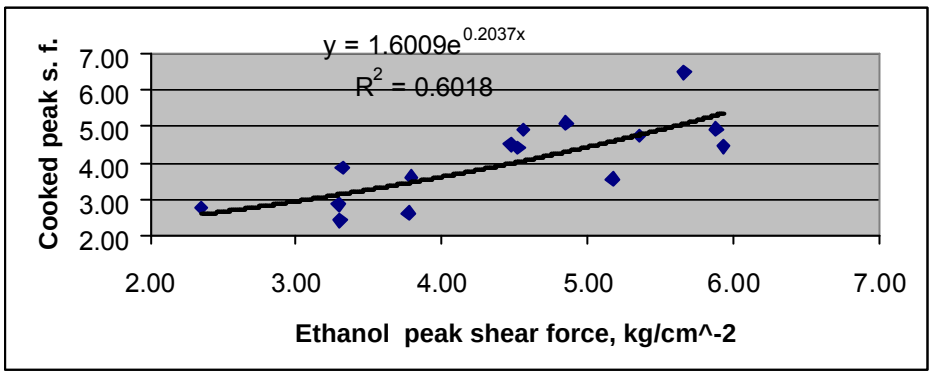

Figure 2. Regression between the shear force in cooked samples (Y) and the shear force in samples treated with ethanol (X)
Note: X = Shear force (kg*cm^2) in cooked ∅ 0.5' LL muscle; Y = Shear force in ∅ 0.5' from an ethanol 2-day treated ∅ 1' sample

The freeze-drying process, although carried out in a single round, in general amplified both the replication and the fasting effects, except for the LL site. On the contrary, the alcohol treatment decreased both the two investigated effects, with the exception of the replication in LL. These findings agree with the other paper (MASOERO *et al*, 2004), where the replication effects were more clearly perceived in FT-NIR spectra of freeze-

dried blood plasma (0.87) than in samples treated with ethanol (0.59), while the fasting effect was very barely perceived (0.10) only after the alcohol reaction.

Table 2. Meat quality and selected body traits: 1-VR chemometrics by FT-NIR of the untreated and treated muscles

	OA	OA_e	OA_gf	LL	LL_e	LL_if	LL_gf	HL	HL_if	HL_gf
Average number of calibrated samples	29.6	28.9	29.3	29.5	29.1	29.7	29.2	29.7	29.9	28.8
1- Replication: *av.0.52*	0.59	0.27	0.82	0.35	0.54	0.86	0.08	0	0.83	0.86
2- Fasting: *av. 0.12*	0.10	0.05	0.15	0.16	0.12	0	0.07	0.11	0.42	0.06
3- pH	0.03	0.26	0.13	0.12	0.40	0.57	0.17	0.36	0.24	0.37
4- L*	0.04	0.08	0	0.10	0.15	0	0.34	0.24	0.36	0.54
5- a*	0	0	0.15	0.05	0	0.06	0.33	0.13	0.05	0
6- b*	0	0	0	0	0	0	0.19	0.25	0.17	0.10
7- C*	0	0	0	0	0.34	0.26	0.41	0.15	0	0
8- H*	0	0	0.07	0	0	0	0.22	0.05	0.32	0.06
9- Cooking losses	0	0.05	0.08	0.21	0.12	0.16	0	0.00	0.06	0.41
10- Shear force in the cooked LL	0	0.04	0	0	0.24	0.28	0.11	0.25	0.10	0.07
11- Shear force in the ethanol LL_e	0	0.38	0	0	0.00	0.22	0	0	0.06	0
12- Water LL	0.41	0.19	0.59	0.20	0.38	0	0.53	0.16	0.42	0.68
13- Dry matter LL_e	0.11	0.10	0.17	0.26	0.69	0.20	0.18	0.22	0.19	0.28
14- Water LL by FD	0.30	0.08	0.66	0.15	0.43	0.09	0.75	0.30	0.32	0.52
15- Water OA	0.52	0.32	0.81	0.26	0	0	0.53	0	0.68	0.75
16- Dry matter OA_e	0.54	0.64	0.88	0	0.29	0.07	0.31	0.16	0.57	0.76
17- Water OA by FD	0.48	0.37	0.95	0.12	0.32	0.14	0.32	0.02	0.75	0.63
18- Water HL by FD	0.48	0.28	0.63	0.09	0	0.23	0.65	0.45	0.45	0.89
19- Estimated LL lipids	0.12	0.08	0.15	0.16	0.41	0.57	r	0.23	0.35	0.45
20- Estim. OA lipids	0.55	0.22	r	0	0.26	0.05	0.54	0.10	0.56	0.70
21- Estim. HL lipids	0.63	0.10	0.61	0	0.02	0.13	0.48	0.53	0.67	r
22- Muscle/bone HL	0.39	0.01	0.11	0.02	0.24	0.18	0.03	0	0.22	0.48

_e = ethanol 95%; _if = intact freeze-dried; _gf = ground freeze-dried; negative values of 1-VR were set to zero; r = reference spectra for lipid estimation with R^2=1

The estimation of the intramuscular lipid content by scanning the intact muscles was nearly half-efficient *vs.* ground freeze-dried tissue for OA and for HL. Nevertheless, in the case of LL, the efficiency was only 0.16. The high value of 0.63 for lipids in HL estimated from intact OA was noteworthy. The treatment in ethanol enhanced the predictability of the original lipids in the FT-NIR spectra of treated tissue when the content was low (LL, 1-VR=0.41), but it became an obstacle for a higher lipid content (OA, 0.22): the lipids can effectively be made solute by the ethanol treatment. The estimation of dry matter content showed a typical trend in all the muscles under

examination: the prediction was minimum for the raw tissue (0.20; 0.52; 0.45 for LL, OA, HL), then it increased for ethanol treated LL and OA (0.69; 0.64), and it was maximum for the three ground freeze-dried muscles (0.75; 0.95; 0.89). The latter high findings completely depend on the indirect negative water-lipid ontogenetic correlation, because the FT-NIR cannot really observe water in this dried tissue. If we refer to the water in the original samples the relative efficiency of the scan of the intact tissue *vs.* the scan of the freeze-dried tissue was 38%, 64% and 51%, respectively.
The meat quality traits were generally badly represented by the NIR spectra. On average, from the four preparations of LL, the 1-VR values were 0.32 for pH, 0.25 for Chroma and 0.15 for Lightness. The NIR examination of intact OA appeared to be suitable for indications on the meat/bone in HL (0.39), whereas alcohol LL gave poor information on this meatiness trait (0.24) and also on the colour parameter (C: 0.34 and L: 0.15), a kind of trait that is easily directly recorded in the carcass with suitable instruments. Similar results for meatiness had previously also been observed in this study for the plasma (MASOERO *et al*, 2004), a sampling method with a higher intrinsic predictability value, if confirmed in an extended data-set.

CONCLUSIONS

The examination of this numerically limited (32), but frequently repeated test (32x10 substrates), allows some conclusions to be made. Direct spectroscopy of intact wet tissue is less informative than NIR analyses of dried tissue, as far as the chemical composition is concerned; the estimation of the intramuscular lipid content is in fact estimated with half accuracy. The loss of information, however, can be balanced by the speed of the process and mainly by the possibility to anticipate the experimental (fasting) or noising (progressive replications) effects even before starting each analysis. The OA localisation appears to be suitable to anticipate the hypothesised and studied factors, as well as some unexpected noising effects, *i.e.* replications. The LL sample treated in ethanol, which can easily be transported to a laboratory, seems to be suitable for tenderness measurements, while the NIR scan should be further investigated for meat quality assessment.

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